

## Immunomodulatory Efficiency of Cow Urine Distillate (CUD) on the Haematology of *Oreochromis mossambicus* (Peters)

### Publication History

Received: 12 March 2015

Accepted: 27 March 2015

Published: 1 April 2015

### Citation

Mala G, Venkatalakshmi S. Immunomodulatory Efficiency of Cow Urine Distillate (CUD) on the Haematology of *Oreochromis mossambicus* (Peters). *Drug Discovery*, 2015, 10(24), 67-75

# Immunomodulatory Efficiency of Cow Urine Distillate (CUD) on the Haematology of *Oreochromis mossambicus* (Peters)

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## ABSTRACT

Cow, *Bos indicus* is mentioned as the most valuable animal in all Veda and it is called as the mother of all. The composition Cow's excretions, Urine, dung, milk, curd and ghee, the five ingredients together known as Panchagavga is the main ingredient of many of our ayurvedic preparations. From the ancient period cow's urine has been used as a medicine for thousands of years Cow Urine therapy and all traditional practices from Indian systems of medicine have a strong scientific base. Traditional system in medicines, whether from Ayurveda or Siddha or the use of Cow Urine distillate as immunomodulator are based on classical texts and system, practices and product handed down over generations going back to Charaka Sushruta, Vagabhatta, the Ashtangahridaga, and the Samhitas. In Ayurveda Cow Urine is Suggested for improving general health. Cow's Urine also consist of 24 types of salts (Lavana in Sanskrit) They are Sodium, Potassium, Magnesium, list all .It is futile in cases of advanced stages where even modern medicine is lacking in solution.Its application in Aquaculture has not been explored so far.Hence the present study has been aimed to assess the effect of Gir Cow Urine on the haematological Parameters of *Oreochromis mossambicus*(peters).The study revealed that the values of, Peripheral leukocyte count and, Hemoglobin level were significantly increased by 0.1% of Gir Cow Urine Distillate.

**KEYWORDS** : *Aeromonas hydrophila*, Gir Cow Urine Distillate, Haematological Parameters, Immunomodulator, *Oreochromis mossambicus*.

## INTRODUCTION

Cow, *Bos indicus* is mentioned as the most valuable animal in all Veda and it is called as the mother of all (Edwin Jarald *et al.*, 2008). The sacredness of the cow is a central and crucial element in Hindu belief. The cow is supposed to be the living symbol of mother Earth. For the early migrants the cow was an indispensable member of family. As agriculture was the occupation of the migrants, the cow provided them with milk and its products and also necessities of life such as fuel, manure for the farm, etc. (Hassan , 2013).

Gir are the hardiest of high yielders in the world. The Gir is a famous milk cattle breed of India. It has been used in the improvement of other breeds including the Red Sindhi and the Sahiwal. Cattle of the breed are famous for their tolerance to stress conditions and resistance to various tropical diseases. ( Gaur *al et.*,2003). In a cow based economy, dung and urine are the major resource of the cow economy, next only to bullock energy. Given this, that a simple resource like cow urine can have such curative powers is unimaginable. Regarding the benefits of cow urine showcases its multiple uses. The cows' uses in dairying and agricultural purposes such as ploughing, transportation, manure and pest repellents are well-known. Dried cow dung (Gobar) is used prolifically in rural India as fuel for fire and a power resource. Environment friendly Gobar Gas Plants across rural India will help save the ozone layer and prevent global warming. (vahanka *et al.*, 2010).From the ancient period in India ,cow's urine has been used as a medicine In veda cow's urine was compared to the nectar (Rigveda 10.15).

Cow urine is a treasure of medicine, being used even in different ancient medicinal practices for the cure of curable and incurable diseases. The analysis of cow urine has shown to contain nitrogen, sulphur , phosphate, sodium, Manganese, carbolic acid and calcium salts (24 Types), vitamin A, B, C, D, E minerals, lactose, enzyme, creatinine, hormones and gold acids. All of these substances are present in a normal human body and their deficiency leads to disease. At that time, cow urine works as a wonderful remedy as it contains a number of beneficial compounds (Faridap *et al.*,2013).

Scientists & clinicians are facing problems in modern allopathic treatment due to the multiple drug resistance in microorganisms, presence of antibiotic residues in food chain and/or associated allergies and autoimmune disorders in man and animals. (Arunkumar *et al.*, 2010)

Experimentally, it has also been proved that among urine from various species the urine of the Indian cows is most effective for its medicinal properties. Immunomodulation is gaining importance for immunopotentiality in hosts against various infections. The cow urine distillate (CUD) is found to have immunomodulatory effect. (Subha Ganguly, 2013).

Sustainable aquaculture depends upon eco-friendly, economically and socially viable culture system. Organic load is a common problem in aquaculture ecosystem. The recycling of organic wastes for fish culture serves the dual purpose of cleaning the environment and providing economic benefits. The recycling of animal dung wastes in fish ponds, for natural fish production is important in integrated farming and to reduce expenditure on costly feeds and fertilizers which form more than 50% of the total input cost ( Padmapriya and Venkatalakshmi , 2014).

*Aeromonas hydrophila* is a gram negative, facultative rod shaped bacteria belonging to the family Vibrionaceae. It causes hemorrhagic septicemia in warm water fishes like channel catfish, tilapia. It is also associated with Epizootic Ulcerative Syndrome (EUS). The etiology of EUS is not known conclusively but it is well known that *A. hydrophila* is an opportunistic pathogen that contributes to the pathogenesis of the lesions (Costa and Wijeyaratne, 1989; Llobrera and Gacutan, 1987). A major problem in the treatment of *A. hydrophila*, is indeed with most bacterial infections is the development of antibiotic resistance (Mitchell and Plumb, 1980; Venkatalakshmi, 2006).

In addition, recently, the increasing consumer concern about the residues of antibiotics, hormones, growth promoters, and the danger of development of antibiotics resistant strains has led to the use of immunostimulants in aquaculture. By definition, immunostimulants are substances that can enhance the nonspecific defense mechanisms as well as specific immune response if the treatment is followed by infection or vaccination (Anderson, 1992a). Many natural and synthetic substances have been reported that potentiate the fish immune system and increase disease resistance (Venkatalakshmi and Michael 2000 ; Logambal *et al.*, 2000).

Worldwide harvest of farmed tilapia has now surpassed 800,000 metric tons, and tilapia are second only to carps as the most widely farmed freshwater fish in the world. The Nile tilapia (*O. niloticus*) was one of the first fish species cultured. Positive aquacultural characteristics of

tilapia are their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms. ( Pillai, 1988).

Hence the study has been planned to study the Immunomodulatory efficiency of Gir Cow Urine Distillate on haematological parameters. Hence based on these qualities, tilapia is selected as the experimental animal for the present study to assess the immunomodulatory effect of Cow Urine, and to explore its application in aquaculture.

## **MATERIALS AND METHODS**

Tilapia (*Oreochromis mossabicus*) a common fresh water cichlid fish was used for the study. Fish procured in healthy condition from local fish farms were stocked in large fiber tanks. In the Laboratory, the fishes were acclimatized in plastic tubs of (vol.70 lt) capacity for one week. Fish of various sexes weighting (10-20gm) were used in the study. Water was changed for once in two days. Fish were fed ad libitum with feed prepared in our laboratory and stored in sterile container. Excess of feed and feces was removed and keep the experimental tubs clean and the experimental fishes healthy.

### **COW URINE COLLECTION**

Cow Urine was collected early morning (5am) six healthy Gir cows (Tag no 201, 195, 126, 204, 190, 207) free from any infection maintained under medical supervision at Goshala in Sri Vital-Rukmini Samasthan, Govindhapuram. Cow Urine was collected in sterile bottles and was pooled to take sample was transported immediately to the laboratory. It was distilled at 60°C for 2 hours in distillate apparatus.

### **EXPERIMENTAL SETUP**

Four plastic tubs of 24(l) x 43(b) cm size were used for the present experiments, six tilapia fish size (10-20gm) were acclimatized in to each plastic tubs were filled with 70 liters of chlorine free tap water and well. After fish were treated with different concentration of Gir Cow Urine Distillate (**T<sub>1</sub> 0.1% , T<sub>2</sub> 0.01% , T<sub>3</sub> 0.001%**) . A control group was maintained separately without CUD treatment. Water was not changed during this period of seven days . Then all the fish were immunized with 10<sup>8</sup> cells/ fish heat killed *A.hydrophila*.

## **PREPARATIONS OF HEAT KILLED *Aeromonas hydrophila***

Overnight culture of *A. hydrophila* were subjected to 60<sup>0</sup>c for 1 hr in a water bath. The culture were centrifuged at 1500 g for 15 min. The packed cphate ells were washed and the required dose was prepared in phosphate buffered saline (D.Sumuel Sudhakaran *et al.*,2006).

## **SERIAL BLEEDING**

Fish were bled serially without sacrificing from the commom cardinal vein using a 24 gauge needle fitted in Glassovan syriage . Blood was collected in sterilized , heparinized vials. The Peripheral and hemoglobin were estimated on the 0<sup>th</sup> ,4<sup>th</sup> ,8<sup>th</sup> , 12<sup>th</sup> and 16<sup>th</sup> days of Post immunization.

## **PERIPHERAL BLOOD LEUKOCYTE COUNT**

Total Peripheral blood leukocytes was counted in a Neubauer counting chamber Using WBC diluting Turk fluied . (Baker F. J and Silverton 1982) .

## **HAEMOGLOBIN ESTIMATION**

It was done by using Sahli's method (Sahli ,1962).

## **RESULTS:**

Fig 1 and 2 reveals that the Cow Urine Distillate has significant effect on the Peripheral blood leukocyte count and Haemoglobin level . The T<sub>1</sub>(0.1% concentration) has elevated the maximum number of cells Peripheral blood leukocyte count and haemoglobin level and T<sub>3</sub>(0.001%) has the minimum effect. The graphs clearly shows that *A.hydrophila* infection has lowered the number of Peripheral blood leukocyte count & heamoglobin level in control where as the Cow Urine Distillate has rectified the effect of *A.hydrophila* infection. T<sub>3</sub> though not elevated the number of cells , it maintains the normal Peripheral and haemoglobin level in the fish .Where as T<sub>1</sub> in addition to relief, it has increased the number of cells and haemoglobin and proves immunostimulation . Hence T<sub>1</sub> could be considered as the optimal dose for immunostimulation.

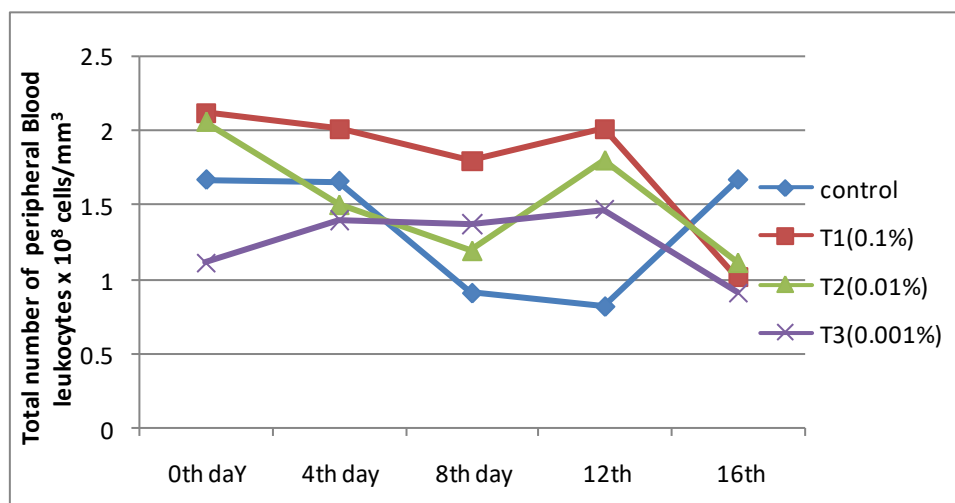
## DISCUSSION:

Conversely, argumentation of immune response has been a matter of much interest among scientists of Veterinary as well as medical sciences in order to increase the all host disease resistance (Chauhan., 2010). Recently the same trend is applicable for aquaculture also. Non specific immunomodulation implies for a more generalized change in immune responsiveness leading to altered host reactivity to many different antigens. The preliminary studies on immunomodulation with cow urine and cow urine distillate was found in mice. It also increases the phagocytic activity of macrophages and secretion of interleukin 1 and 2 from the peripheral blood leukocytes (Chauhan, 2001a & b and Kumar et al., 2002). They have reported increase in humoral and cellular immunity of 45% and 59% respectively. The increase in number of peripheral blood leukocytes in the present study is in line with the previous works done in mice (Chauhan, 2011) and in rabbits (Ankita Joshi et al., 2012). Hence the indigenous cattle scientifically called as *Bos indicus* which is thought to be the world's oldest domesticated cattle is now proved for its superiority in therapeutic aspects. Its immense potential as an immunomodulator has been proved in the present study.

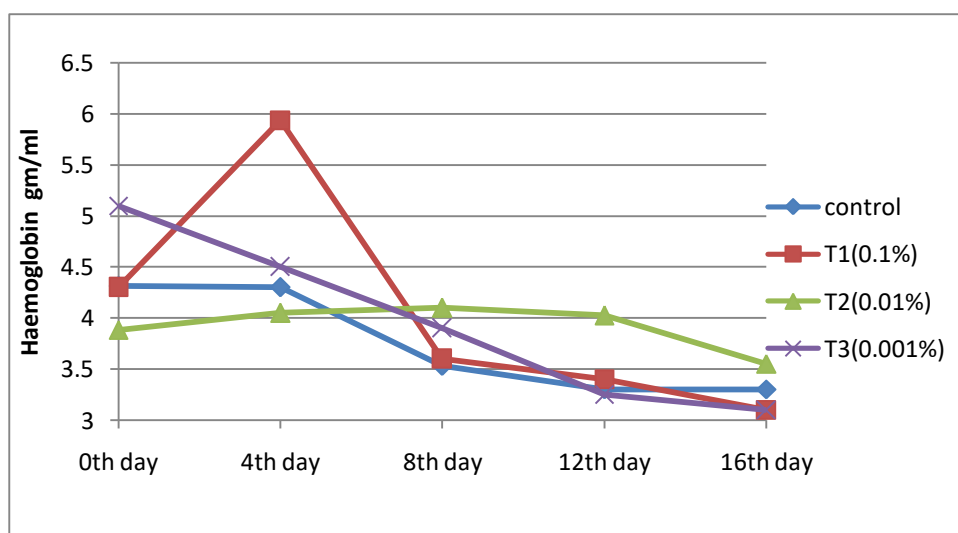
## CONCLUSION

Gir Cow Urine distillate (GCUD) possesses immunomodulatory effect was judged by increased Peripheral blood leukocyte count and Haemoglobin level (HB) in *Oreochromis mossambicus* (Peters). Hence the use of Cow Urine Distillate as an immunomodulating agent at proper dose level may be advocated.

**Fig 1. The effect of Cow Urine Distillate (CUD) on Peripheral Bloodleukocyte count of *Oreochromis mossambicus***



**Fig 2. The effect of Cow Urine Distillate (CUD) on Haemoglobin level of *Oreochromis mossambicus***



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